Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Decoupling of Acetylcholine Influx and Theta Power in the Hippocampus

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Review of Zhang et al.

The importance of acetylcholine (ACh) in higher cognitive functions has been postulated for decades (Sarter et al., 2005). Attention, arousal, novelty detection, and learning all rely on an intact cholinergic system, which arises principally from the nucleus basalis and the medial septum. Traditionally, pharmacological blockade and transmitter-specific lesions have been used to determine the involvement of ACh in neural processes. Later, microdialysis was used to provide a robust measure of the amount of neurotransmitter released in the area of interest with low temporal resolution (i.e., minutes). However, cognitive processes such as attention and arousal, and physiological processes like network rhythms, dynamically change in less than a second. Recent improvements of amperometric recording techniques allow fast, subsecond measurement of extracellular ACh concentration in the brain *in vivo* (Parikh et al., 2007; Burmeister et al., 2008). With these new tools, old theories regarding the cholinergic system and brain function can now be tested using

more appropriate physiological time scales (<1 s).

Hippocampal theta oscillations have been implicated in many cognitive processes such as emotion, reward, learning, and memory (Buzsáki, 2005). In intact animals, hippocampal theta oscillations are known to be modulated by cholinergic, GABAergic, and glutamatergic innervations from the medial septum. The nature of ACh involvement in hippocampal theta oscillations is controversial. Whereas earlier studies postulated that ACh input to the hippocampus rhythmically pace theta oscillations (Stewart and Fox, 1990), it is clear from more recent studies that septal cholinergic neurons exhibit slow, nonrhythmic activities and thus are unable to rhythmically drive hippocampal theta oscillations (Simon et al., 2006). Lesion studies suggest septal ACh input to the hippocampus is more likely to play a role in the modulation of hippocampal theta power in freely moving animals (Lee et al., 1994) but were demonstrated to be indispensable for hippocampal theta generation under anesthesia (Kramis et al., 1975; Yoder and Pang, 2005). To critically address the role of ACh in hippocampal theta oscillations, a direct demonstration of simultaneous changes in hippocampal field activity and ACh concentration is necessary. Based on previous work, it would be predicted that under anesthesia (where theta is cholinergic-dependent), ACh concentration should covary with hippocampal theta power. Recent work published in *The Jour-*

nal of Neuroscience by Zhang et al. (2010) used a novel amperometric technique to concurrently record CA1 field potentials and choline concentration in the urethane-anesthetized rat to address the temporal relationships between theta power and ACh concentration in the hippocampus, furthering our understanding of the role ACh in hippocampal theta oscillations.

The use of *in vivo* amperometry by Zhang and colleagues (2010) provided the ability to record neurochemical (<0.5 Hz) and neurophysiological (>0.5 Hz) signals concurrently by high-pass filtering of an electrical signal obtained by a single sensor (their Fig. 1). The concentration of ACh was assessed by the measurement of choline, the metabolite of ACh. The measurement of choline concentration was made possible by coating the amperometry sensors with choline oxidase and a size-exclusion polymer to limit the signal from potential electroactive interferents. Electrons donated from the oxidation of choline through the H₂O₂ intermediate is detected as a current at the recording site held at a positive potential (Bruno et al., 2006). It is important to note that this method of estimating ACh concentration assumes that all ACh released into CA1 is metabolized to choline by the extremely effective acetylcholine esterase (AChE), but does not take into account possible direct choline releases or concentration of preexisting choline.

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By recording from multiple depths within CA1, Zhang and colleagues (2010) localized the largest changes in choline signals to the strata oriens/pyramidale (their Fig. 3), where most of the cholinergic fibers and AChE activity are found (Nyakas et al., 1987). The slow fluctuations in choline signals were correlated to both spontaneously occurring and tail-pinch-induced CA1 theta oscillations with a long latency to peak (their Fig. 2). To address the question of whether ACh release is involved in CA1 theta initiation, Zhang and colleagues (2010) examined the temporal relationships between the choline signal and theta power using the cross-correlation technique. Based on these measures, the time to peak for the choline signal was found to lag behind the time to peak for CA1 theta power by 20–50 s (their Fig. 4), prompting the authors to conclude that ACh is not required for CA1 theta generation. This is an unexpected conclusion because hippocampal theta oscillations in urethane-anesthetized rats have previously been shown to require ACh (Kramis et al., 1975; Yoder and Pang, 2005). The proposal made by Zhang et al. (2010) seems to be based on the assumption that the ACh release in CA1 needs to exhibit the same time course as the simultaneously recorded theta power for ACh to participate in theta generation. This estimate does not take into account the choline already in CA1 because the particular coating technique can only detect changes in choline concentration, not its absolute concentration. If the absolute required ACh concentration in the CA1 is substantially lower than the peak choline concentration detected, the slight but steady increase of ACh at the onset of CA1 theta oscillations (their Figs. 2A, 4A, 5F) may in fact be sufficient for its contribution to generating CA1 theta.

Because the dynamics of ACh release in the hippocampus are predicted to arise from the activity of medial septal cholinergic neurons, Zhang et al. (2010) recorded the activity of septal neurons under the same conditions that hippocampal choline concentration was measured. Previous work has shown that slow-firing septal neurons are most likely to be cholinergic, whereas fast-firing neurons are most likely GABAergic (Stewart and Fox, 1990; Simon et al., 2006). Zhang et al. (2010) found that the time course of the slow-firing septal cells had a temporal profile similar to that of choline concentration, supporting the notion that septal cholinergic neurons are indeed responsible for the changes in choline concentration observed in the postsynaptic CA1 region (their Fig. 5). Fast-spiking neurons (presumably medial septal interneurons)

showed a stronger temporal correlation with the emergence of theta oscillations (Zhang et al., 2010, their Fig. 5F), supporting previous claims that medial septal interneurons are involved in hippocampal theta initiation (Hangya et al., 2009). Intriguingly, it is clearly shown in Zhang et al.'s (2010) Figure 5, A, C, F, that the putative medial septal cholinergic neurons, as a population, do in fact increase their firing rate at the onset of CA1 theta oscillations with firing rates rapidly ramping above baseline activity (their Fig. 5C). This contrasts with recorded CA1 choline concentration (their Fig. 5F), suggesting decoupling between putative septal cholinergic neuron firing rate and CA1 choline concentration around CA1 theta onset. It may be possible that, because Zhang et al. (2010) were detecting choline, the initial ACh increase in the CA1 was masked by a high proportion of receptor-bound ACh and the relatively low AChE activity in the CA1 (Perrier et al., 2003) and fast choline uptake. Saturation of receptor-bound ACh and choline uptake may eventually result in the subsequent increased temporal coupling between medial septal cholinergic activity and choline concentration in the CA1 (Zhang et al., 2010, their Fig. 5F). Direct measurements of ACh concurrent with choline (Burmeister et al., 2008) may clarify the nature of observed decoupling between choline concentration and putative medial septal cholinergic neuron firing rate.

Although it remains unclear whether ACh is necessary to initiate hippocampal theta in urethane-anesthetized animals, it is clear that the choline concentration in CA1 is decoupled from theta power recorded from the same region. What then, may be the functional significance of slow ACh transients reported by Zhang et al. (2010) if they are not related to the generation of hippocampal theta or the modulation of its power? In freely moving animals, it is thought that the presentation of novel stimuli elicits high hippocampal ACh to suppress excitatory intrahippocampal pathways (within CA3 and between CA3 and CA1) and facilitate entorhinal cortex inputs to encourage encoding, whereas low ACh may promote retrieval by releasing CA1/CA3 inhibition (Hasselmo, 2006). Therefore, phasic hippocampal ACh transients may play a major role in coordinating memory encoding and retrieval states. With the techniques described by Zhang et al. (2010), it is possible to directly examine how changes in ACh concentration and hippocampal network activity may vary according to behavior, thereby providing insights to the role of ACh in hippocampal function.

In conclusion, Zhang et al. (2010) provide important insights regarding changes in hippocampal ACh concentrations during the initiation and termination of CA1 theta rhythms in urethane-anesthetized rats. Using improved amperometric recording technology, the authors simultaneously measured local field potentials and estimated ACh concentration with high temporal resolution. Although the time and rate of change of CA1 choline concentration and theta power were not strongly correlated, it remains unclear whether choline measurements used by Zhang et al. (2010) are indeed representative of CA1 ACh concentration, and how much ACh release by septal cholinergic neurons may be required for hippocampal theta generation under urethane anesthesia. Further research should determine how ACh transients are regulated, as well as determining the biological significance of the observed slowly ramping ACh release on hippocampal circuitry. These electrochemical techniques should pave the way for future studies of hippocampal ACh function in freely moving animals. Therefore, Zhang et al. (2010) provide an important first step into a new age of experiments to reevaluate the complex relationship between ACh and memory systems.

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